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(54) **ANALGESIC ANTITUMOR PEPTIDE FROM SCORPION AND METHOD OF PRODUCING IT**

ANALGETISCHES ANTITUMORPEPTID AUS SKORPIONEN UND VERFAHREN ZU DESSEN HERSTELLUNG

PEPTIDE PROVENANT DU SCORPION AYANT UN EFFET ANALGESIQUE ET ANTICANCEREUX ET SON PROCEDE D'OBTENTION

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to the field of biomedicine technology. In particularly, the present invention provides a method for the preparation of a peptide having analgesic and antitumor activity from scorpion.

BACKGROUND OF THE INVENTION

[0002] Scorpion is also named as quan chong scorpion and quan xie scorpion, whose pharmacological property is described in detail in the traditional medical famous works such as "shi jing", "kai bao ben cao" and "ben cao gang mu", and so on. Scorpion has the function of dispelling rheumatism, calming affright, activate the channels, alleviating pains, attacking toxin and dispersing, which is used to treat febrile convulsion, convulsion, stroke, hemiplegia, rheumatism, hemicrania, headache, neuralgia, body pain, tumefaction and various kinds of malignancies. However, it is not clear which component in scorpion plays the function of activate the channels, relieving pains, attacking toxin and dispersing.

DISCLOSURE OF THE INVENTION

[0003] The peptide having analgesic and antitumor activity according to the method of the present invention is screened, isolated, purified, found and obtained by a chemical method including steps of extraction, chromatography, ultra-filtration, purification, etc. from scorpion or scorpion venom The peptide having analgesic and antitumor activity not only can be purified and prepared from scorpion or scorpion venom, but also can be expressed and obtained by recombinant DNA technology, namely gene engineering method. The peptide having analgesic and antitumor activity also can be obtained by chemical synthesis method. The peptide has a significant biological activity of analgesic and antitumor.

[0004] The present invention provides a peptide having analgesic and antitumor activity derived from scorpion, wherein the amino acid sequence of the peptide is shown as SEQ ID NO: 1. The present invention also provides partial fragment, derivative or analogue of the peptide having analgesic and antitumor activity, wherein the partial fragment or analogue or derivative is obtained by deleting the partial amino acids of N terminal or C terminal of the peptide, or by substituting, adding or deleting one or more amino acids or the partial amino acids in the extended part of N terminal or C terminal of the peptide. In one embodiment, the present invention provides an analogue of the peptide having analgesic and antitumor activity, wherein the amino acid sequence is shown as SEQ ID NO: 3. In another embodiment, the present invention provides a derivative of the peptide having analgesic and antitumor activity, wherein the amino acid sequence is shown as SEQ ID NO: 4.

[0005] The present invention provides a method for the preparation of the peptide having analgesic and antitumor activity derived from scorpion, or partial fragment, derivative or analogue thereof, wherein the amino acid sequence of the said peptide is shown as SEQ ID NO: 1, which is isolated from the scorpion or scorpion venom, comprising the following steps:

(1) extracting scorpion or scorpion venom with distilled water, acidic solution and basic solution, respectively, then centrifuging to remove the impunity,
(2) performing hydrophobic chromatography of the extract to give the active ingredient having analgesic and antitumor activity,
(3) ultra-filtrating the active ingredient having analgesic and antitumor activity to remove salt and impurity proteins, concentrating, purifying the active ingredient having analgesic and antitumor activity by cation exchange chromatography,
(4) further purifying the active ingredient having analgesic and antitumor activity by hydrophobic chromatography,
(5) desalting by ultra-filtration and concentrating, and purifying by gel filtration chromatography to give electrophoresis pure active ingredient having analgesic and antitumor activity,
(6) further purifying the active ingredient having analgesic and antitumor activity by reverse phase column chromatography.

[0006] According to one preferred embodiment of the present invention, wherein
in step (1), the scorpion venom is extracted by distilled water, then the extract is extracted by the acidic solution with a pH of 2 or more, and the basic solution with a pH of 12 or less to remove the impunity;
in step (2), hydrophobic chromatography is performed twice, wherein the chromatography medium is phenyl-, octane- or butyl- hydrophobic chromatography medium, the pH of sodium phosphate eluent is physiologically acceptable, wherein the concentration of phosphate radical is 5mM-100mM, and the gradient of concentration of $(NH_4)_2SO_4$ or Na_2SO_4 is 2.0M-0.0M;

in step (3) and (5), the trapped molecular weight of ultra-filtration membrane is 30kDa or 1 kDa;
in step (5), the medium of gel filtration chromatography is chromatographic filler Sephacry1 S-100Hr, Superose 6 prep grade, Superose 12 prep grade, Superdex 30 prep grade, or Superdex 75 prep grade, and the pH of eluent is in a range of between 2 and 12;
in step (6), the medium of reverse phase chromatography is chromatographic filler SOURCE 15RPC or SOURCE 30RPC, the eluent is 0.1% trifluoroacetic acid-acetonitrile aqueous solution, and the gradient of concentration of acetonitrile is from 20% to 95%; the whole process including extraction, isolation and purification is performed in the half-sterile or sterile room at 10°C-30°C.

[0007] According to another preferred embodiment of the present invention, wherein
in step (1), the scorpion is homogenized and extracted by distilled water, then the extract is extracted by the acidic solution with a pH of 2 or more, and the basic solution with a pH of 12 or less to remove the impunity;
in step (2), hydrophobic chromatography is performed twice, wherein the chromatography medium is phenyl-, octane- or butyl- hydrophobic chromatography medium, the pH of sodium phosphate eluent is physiologically acceptable, wherein the concentration of phosphate radical is 5mM-100mM, and the gradient of concentration of $(NH_4)_2SO_4$ or Na_2SO_4 is 2.0M-0.0M;
in step (3) and (5), the trapped molecular weight of ultra-filtration membrane is 30kDa or 1 kDa;
in step (5), the medium of gel filtration chromatography is chromatographic filler Sephacry1 S-100Hr, Superose 6 prep grade, Superose 12 prep grade, Superdex 30 prep grade, or Superdex 75 prep grade, and the pH of eluent is in a range of between 2 and 12;
in step (6), the medium of reverse phase chromatography is chromatographic filler SOURCE 15RPC or SOURCE 30RPC, the eluent is 0.1% trifluoroacetic acid-acetonitrile aqueous solution, and the gradient of concentration of acetonitrile is from 20% to 95%; the whole process including extraction, isolation and purification is performed in the half-sterile or sterile room at 10°C-30°C.

[0008] The present invention also provides a method for the preparation of the peptide or partial fragment, derivative or analogue thereof having analgesic and antitumor activity according to the present invention, which is synthesized, processed, purified and prepared by chemical synthesis method.

[0009] The present invention further provides a method for the preparation of the peptide or partial fragment, derivative or analogue thereof having analgesic and antitumor activity according to the present invention, which is expressed, processed, purified and prepared by recombinant DNA technology.

[0010] The present invention also provides use of the peptide or partial fragment, derivative or analogue thereof having analgesic and antitumor activity wherein the amino acid sequence of the said peptide is selected in the group consisting of SEQ ID NO. 1, SEQ ID NO:3, SEQ ID NO: 4, in the preparation of analgesic medicament. The present invention further provides use of the peptide or partial fragment, derivative or analogue thereof wherein the amino acid sequence of the said peptide is selected in the group consisting of SEQ ID NO. 1, SEQ ID NO:3, SEQ ID NO: 4, having analgesic and antitumor activity in the preparation of antitumor medicament.

[0011] The present invention is achieved by the following technical schemes:

[0012] According to the differences in molecular weight, isoelectric point, the hydrophobic properties of molecular surface between the peptide having analgesic and antitumor activity derived from the scorpion or scorpion venom and other proteins, polypeptide, or enzymes, the peptide having analgesic and antitumor activity derived from the scorpion, wherein the amino acid sequence is shown as SEQ ID NO: 1, is extracted, purified and obtained from the scorpion or scorpion venom by ultra filtration, gel filtration chromatography, ion exchange chromatography, hydrophobic chromatography and reverse phase chromatography. The extraction, isolation and purification processes of the peptide having analgesic and antitumor activity are performed in the half-sterile or sterile room at 10°C-30°C. According to the structure of the active peptide, the peptide or the partial fragment thereof or derivative or analogue thereof having analgesic and antitumor activity is obtained by the chemical synthesis method. According to the structure of the active peptide, the gene of the peptide or the partial fragment thereof or derivative or analogue thereof having analgesic and antitumor activity is obtained by the chemical synthesis method or biotechnology. The gene is cloned into an engineering expression vector by recombinant DNA technology, i.e. gene engineering method. The peptide or the partial fragment thereof or derivative or analogue thereof having analgesic and antitumor activity is obtained by expression and purification.

[0013] The peptide having analgesic and antitumor activity from scorpion according to the present invention can be prepared into various kinds of conventional formulations according to the traditional preparation technology in the field. The example of formulation is injectable formulation, such as intravenous injection and intramuscular injection, oral administration formulation. The oral administration formulation should be a formulation that can not be degraded by pepsin, such as inclusions. The administration amount is determined according to the administration route, the condition of the subject and other factors. Typically, for adults with about 70Kg weight, preferably the dose is about 0.01-100 mg/kg weight. When required, the upper limit of dose can be exceeded.

[0014] The peptide or the partial fragment thereof or derivative or analogue thereof having analgesic and antitumor activity according to the present invention not only can be used as the drug for treating various kinds of pains and tumor

diseases, but also can be used as the research tool to screen the new analgesic and antitumor compounds.

[0015] The method for the preparation of the peptide or the partial fragment thereof or derivative or analogue thereof having analgesic and antitumor activity has the advantage of simple and high yield, which has guide significance and utility value to research and develop a new analgesic and antitumor medicine, and establishes a basis of industrialization production.

EXAMPLES

Example 1:

[0016] 2.0g of scorpion venom was dissolved into 20ml distilled water, centrifuged at 15000 rpm/min for 15 min, the supernatant was adjusted to pH 2 with 0.01 M HCl or phosphoric acid and centrifuged at 15000 rpm/min for 15 min, then the supernatant was adjusted to pH 12 using sodium phosphate and centrifuged at 15000 rpm/min for 15 min. The pH of supernatant was adjusted to physiologically acceptable pH with HCl or phosphoric acid, $(NH_4)_2SO_4$ or Na_2SO_4 was added to the concentration of 2M, then loaded onto the hydrophobic chromatography column (2.6cm×30cm), such as phenyl- or octane- hydrophobic chromatography column, balanced with 2M $(NH_4)_2SO_4$ or Na_2SO_4 solution in advance, then eluted with 1.5M, 1.0M and 0.5M $(NH_4)_2SO_4$ or Na_2SO_4 buffers respectively, finally eluted with 50mM sodium phosphate buffer (pH was adjusted to physiologically acceptable pH). The elution solutions containing the analgesic and antitumor active ingredients were combined, then performed ultra filtration using the ultra-filtration membranes with trapped the molecular weight of 30kDa (kilo Daltons) and 1 kDa respectively to remove the salts and impurity proteins in the elution solution, finally balanced with 10mM pH4.0 citric acid-sodium phosphate. Then the balanced ultra-filtration sample in the range of 30kDa and 1kDa (kilo Daltons) was loaded onto the cation exchange column (2.6cm×50cm), such as CM- and SP- cation exchange chromatography column, balanced with 10mM pH4.0 citric acid-sodium phosphate buffer in advance. At first the column was thoroughly washed with 10mM pH4.0 citric acid-sodium phosphate buffer, then performed gradient elution of NaCl solution (0.0M-1.0M) by using 10mM pH4.0 citric acid-sodium phosphate buffer as the basic buffer. The elution solutions containing the analgesic and antitumor active ingredients were combined, adjusted pH to physiologically acceptable pH with NaOH or HCl or phosphoric acid, then $(NH_4)_2SO_4$ or Na_2SO_4 was added to 2.0M, loaded onto the hydrophobic chromatography column (2.6cm×30cm), such as butyl- or octane- hydrophobic chromatography column, balanced with 2M $(NH_4)_2SO_4$ or Na_2SO_4 solution in advance, then performed gradient elution of the neutralized salt (2.0M-0.0M) by using 50mM sodium phosphate (pH was adjusted to physiologically acceptable pH) as the basic solution. The second peak of two eluting peaks was combined and collected, ultra-filtrated with the ultra-filtration membrane of the trapped molecular weight of 1kDa (kilo Daltons), the sample was loaded onto the gel filtration chromatography column (1.6cm×60cm), such as Sephacry1 S-100Hr or Superose6, 12prep grade or Superdex30, 75prep grade chromatography column, balanced with 20mM sodium phosphate (pH was adjusted to physiologically acceptable pH, containing 150mM NaCl) in advance. The peak ingredients with the largest eluting peak area were combined and collected, the ingredient was a single silver-stained band in SDS-PAGE, the active ingredient obtained in gel filtration chromatography was loaded onto the reverse phase column (1.0cm×15cm), such as SURCE 15RPC and 30PRC chromatography column, balanced with 0.1% trifluoroacetic acid-20% acetonitrile aqueous solution in advance. At first, the column was thoroughly washed with 0.1% trifluoroacetic acid-20% acetonitrile aqueous solution, then performed gradient elution with acetonitrile (20% to 95%). The peak ingredients with the largest eluting peak area were combined and collected. The eluting peak area of the ingredient was more than 95% of the whole eluting peak area of the reverse phase chromatography. The ingredient was collected and dried in vacuum at the temperature lower than 30°C (lyophylization in vacuum also can be used). The traditional reducing agent was used to open and protect the disulfide bond of the peptide having analgesic and antitumor activity derived from scorpion. The N-terminal amino acid sequence of the peptide was determined according to Edman degradation method by applying the protein sequencer of Applied Biosystems. The C- terminal amino acid sequence of the peptide was determined by carboxypeptidase Y degradation method. The amino acid sequence of the peptide having analgesic and antitumor activity derived from scorpion was shown as SEQ ID NO: 1.

Example 2:

[0017] 1000g scorpion was homogenized by adding 2.5L distilled water, and centrifuged at 4500 rpm/min for 20 min. The supernatant was collected. The precipitate was homogenized by adding 1.5L of distilled water, and centrifuged at 4500 rpm/min for 20 min. The supernatant was collected. The precipitate was homogenized by adding 1.0L of distilled water, and centrifuged at 4500 rpm/min for 20 min. The supernatant was collected, the extracts of the above three times was combined and adjusted pH to 2 using 0.01M HCl or phosphoric acid, centrifuged at 5000 rpm/min for 15 min. The supernatant was collected and adjusted pH to 12 using sodium phosphate, then centrifuged at 5000 rpm/min for 15 min. Other processes were the same as those in Example 1.

Example 3:

[0018] The fragment of nucleic acid was synthesized according to the amino acid sequence of the peptide having analgesic and antitumor activity derived from scorpion (the amino acid sequence was shown as SEQ ID NO: 1). The full-length gene of the peptide or the gene fragment of the derivative or analogue thereof or the partial gene fragments thereof having analgesic and antitumor activity were screened by hybridization from the scorpion cDNA pool or cDNA library or DNA library. For example, the full-length gene sequence of the peptide derived from the breeding scorpion was shown as SEQ ID NO: 2. The full-length gene sequence of peptide derived from the wild scorpion was identical with that from breeding scorpion except that the codons encoding the amino acids of position 24 and position 29 were GAC and AAT respectively.

Example 4:

[0019] The PCR degenerate primers were designed according to the amino acid sequence of N- terminal or C-terminal of the peptide having analgesic and antitumor activity derived from scorpion. Primer 1: 5'-GGAATTCCATGGTACGCGAT-GGTTATAT-3'(GTA CGC GAT GGT TAT encoded the amino acids of position 1-5 of the peptide) (SEQ ID NO: 5). Primer 2: 5'-TTGCTCGAGACCG CCATTGCATTTTGG-3' (ACC GCC ATT GCA TTT TGG was the sequence of the complementary strand encoding the amino acids of position 61-66 of the peptide) (SEQ ID NO:6). The analogue of the recombinant peptide was obtained from these two primers, wherein the amino acid sequence was shown as SEQ ID NO:3. Primer 3: 5'-CAAGCATATGGTACGCGATGGTTATAT-3' (GTA CGC GAT GGT TAT encoded the amino acids of position 1-5 of the peptide) (SEQ ID NO: 7), primer 4: 5'-ATAGGATCCTAATTAACCGCCATTGCATTTTGG-3' (ACC GCC ATT GCA TTT TGG encoded the sequence of the complementary strand of the amino acids of position 61-66 of the peptide) (SEQ ID NO:8). The derivative of the recombinant peptide was obtained from these two primers, wherein the amino acid sequence was shown as SEQ ID NO:4. The full-length gene of the peptide or the gene fragment of the derivative or analogue thereof or the partial gene fragments thereof having analgesic and antitumor activity were screened from cDNA pool or cDNA library or DNA library by PCR reaction.

Example 5:

[0020] The full-length gene of the peptide or the gene fragment of the derivative or analogue thereof or the partial gene fragments thereof were synthesized according to the structure (amino acid sequence) of the peptide or partial fragment, derivative or analogue thereof having analgesic and antitumor activity shown as SEQ ID NO:1, 3 and 4 in the specification.

Example 6:

[0021] The genes obtained in Example 3, 4 and 5 were digested by restricted endoenzymes and cloned into various kinds of the engineering expression vectors. For example, the obtained PCR products in Example 4 were digested by restricted endoenzymes NcoI/XhoI or by NdeI/BamHI and cloned into the E.coli expression vector pET28a. The recombinant peptide or the extended or shorted derivative or analogue thereof (the amino acid sequences were shown as SEQ ID NO: 3 and 4) were obtained by expression, post-process, isolation and purification.

Example 7:

[0022] The mouse with acetic acid writhing model was administrated intravenously with the peptide derived from the scorpion or partial fragment, derivative or analogue thereof having analgesic and antitumor activity obtained in Example 1, 2 and 6 in a dose of 0.1mg/kg weight to 16.0mg/kg weight. The inhibitory rate of writhing response of mouse was 0.0% to 100%. For example, the ED_{50} (median effective dose) of the peptide of the SDS-PAGE single band obtained in Example 1 was 0.47mg/kg weight, the ED_{50} (median effective dose) of morphine by intravenous administration as the positive control was 16.0mg/kg weight. The above samples were administrated by stomach infusion in a dose of 0.1mg/kg weight to 16.0mg/kg weight. The inhibitory rate of writhing response of mouse was 0.0% to 100%. For example, the ED_{50} (median effective dose) of the peptide of the SDS-PAGE single band obtained in Example 2 was 2.86mg/kg weight. In the mouse heat plate model, the above samples were administrated by intravenous injection in a dose of 0.1 mg/kg weight to 16.0mg/kg weight. The threshold value of pain of the mouse (the time required to lick the back foot on the heat plate of 55°C±5°C) was the normal value (from 10 seconds to 30 seconds) to 60 seconds (if the pain threshold value was more than 60 seconds, the mouse did not lick the back foot yet, then the mouse was taken out). For example, the peptide of SDS-PAGE single band obtained in example 1 and 2 were injected intravenously at 0.6 mg/kg weight. The pain threshold were raised in a rate of 16.5%, 145 7%, 219.1%, 219.1%, 177.6%, 32.4%, 14.9% at 5min, 15min,

25min, 35min, 45min, 60min and 90min respectively. When morphine was administrated intravenously in a dose of 10.0 mg/kg weight, the pain threshold were raised in a rate of 0.6%, 27.0%, 112.6%, 177.9%, 125.8%, 50.9% and -3 7% at 5min, 15min, 25min, 35min, 45min, 60min and 90min respectively.

Example 8:

[0023] In mouse experimental tumor model, such as ehrlich ascites carcinoma and S180 entity tumor, the peptide derived from the scorpion or partial fragment, derivative or analogue thereof having analgesic and antitumor activity obtained in example 1,2 and 6 was injected subcutaneously in a dose of 0.1mg/kg weight to 16.0mg/kg weight for continuous seven days. The inhibitory rate of tumor weight of S180 sarcoma solid tumor [(C-T)/C, wherein C was the average tumor weight of the control group and T was the average tumor weight of the test group] was 0% to 62%. For example, the peptide of the SDS-PAGE single band obtained in Example 1 was administrated at the dose of 0.639mg/kg weight. The inhibitory rate of tumor weight was 30.9%. The inhibitory rate of tumor weight was 50.5% when cyclophosphamide was used as the positive control in the dose was 60.0mg/kg weight. The peptide derived from the scorpion or partial fragment, derivative or analogue thereof having analgesic and antitumor activity obtained in example 1,2 and 6 was administrated by stomach infusion in a dose of 0.1mg/kg weight to 16.0mg/kg weight for continuous ten days. The rate of life prolonging of mouse with ehrlich ascites carcinoma were 0% to 46%. For example, the rate of life prolonging of the peptide of the SDS-PAGE single band obtained in Example 2 were 16.8% and 31.5% in the dose of 0.213mg/kg weight and 0.639mg/kg weight respectively. For the cyclophosphamide as the positive control, the rate of life prolonging was 15.6% in a dose of 60.0mg/kg weight (administration by subcutaneous injection).

SEQUENCE LISTING

[0024]

<110> Shenyang Pharmaceutical university

<120> A PEPTIDE HAVING ANALGESIC AND ANTITUMOR ACTIVITY FROM SCORPION AND THE METHOD FOR PREPARING THE SAME

<130> FP04005

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Claims

1. A method for the preparation or a peptide having analgesic and antitumor activity derived from the scorpion or partial fragment, derivative or analogue thereof having analgesic and antitumor activity, wherein the amino acid sequence of the said peptide is shown as SEQ ID NO: 1, which is isolated from the scorpion or scorpion venom, comprising the following steps:

   (1) extracting scorpion or scorpion venom with distilled water, acidic solution and basic solution, respectively, then centrifuging to remove the impurity,
   (2) performing hydrophobic chromatography of the extract to give the active ingredient having analgesic and antitumor activity,
   (3) ultra-filtrating the active ingredient having analgesic and antitumor activity to remove salt and impurity proteins, concentrating, purifying the active ingredient having analgesic and antitumor activity by cation exchange chromatography,
   (4) further purifying the active ingredient having analgesic and antitumor activity by hydrophobic chromatography,
   (5) desalting by ultra-filtration and concentrating, and purifying by gel filtration chromatography to give electrophoresis pure active ingredient having analgesic and antitumor activity,
   (6) further purifying the active ingredient having analgesic and antitumor activity by reverse phase column chromatography.

2. The method according to claim 1, wherein
   in step (1), the scorpion venom is extracted by distilled water, then the extract is extracted by the acidic solution with a pH of 2 or more, and the basic solution with a pH of 12 or less to remove the impurity,
   in step (2), hydrophobic chromatography is performed twice, wherein the chromatography medium is phenyl-, octane- or butyl- hydrophobic chromatography medium, the pH of sodium phosphate eluent is physiologically acceptable, wherein the concentration of phosphate radical is 5mM-100mM, and the gradient of concentration of $(NH_4)_2SO_4$ or Na_2SO_4 is 2.0M-0.0M;
   in step (3) and (5), the trapped molecular weight of ultra-filtration membrane is 30kDa or 1kDa;
   in step (5), the medium of gel filtration chromatography is chromatographic filler Sephacry1 S-100Hr, Superose 6 prep grade, Superose 12 prep grade, Superdex 30 prep grade, or Superdex 75 prep grade, and the pH of eluent is in a range of between 2 and 12;
   in step (6), the medium of reverse phase chromatography is chromatographic filler SOURCE 15RPC or SOURCE 30RPC, the eluent is 0.1% trifluoroacetic acid-acetonitrile aqueous solution, and the gradient of concentration of acetonitrile is from 20% to 95%; the whole process including extraction, isolation and purification is performed in the half-sterile or sterile room at 10°C-30°C.

3. The method according to claim 1, wherein
in step (1), the scorpion is homogenized and extracted by distilled water, then the extract is extracted by the acidic solution with a pH of 2 or more, and the basic solution with a pH of 12 or less to remove the impurity;
in step (2), hydrophobic chromatography is performed twice, wherein the chromatography medium is phenyl-, octane- or butyl- hydrophobic chromatography medium, the pH of sodium phosphate eluent is physiologically acceptable, wherein the concentration of phosphate radical is 5mM-100mM, and the gradient of concentration of $(NH_4)_2SO_4$ or Na_2SO_4 is 2.0M-0.0M;
in step (3) and (5), the trapped molecular weight of ultra-filtration membrane is 30kDa or 1 kDa;
in step (5), the medium of gel filtration chromatography is chromatographic filler Sephacry1 S-100Hr, Superose 6 prep grade, Superose 12 prep grade, Superdex 30 prep grade, or Superdex 75 prep grade, and the pH of eluent is in a range of between 2 and 12;
in step (6), the medium of reverse phase chromatography is chromatographic filler SOURCE 15RPC or SOURCE 30RPC, the eluent is 0.1% trifluoroacetic acid-acetonitrile aqueous solution, and the gradient of concentration of acetonitrile is from 20% to 95%; the whole process including extraction, isolation and purification is performed in the half-sterile or sterile room at 10°C-30°C.

4. A method for the preparation of a peptide having analgesic and antitumor activity derived from the scorpion or partial fragment, derivative or analogue thereof having analgesic and antitumor activity, wherein the amino acid sequence of the said peptide is selected in the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ NO: 4,, which is synthesized, processed, purified and prepared by chemical synthesis method.

5. A method for the preparation of a peptide having analgesic and antitumor activity derived from the scorpion or partial fragment, derivative or analogue thereof having analgesic and antitumor activity, wherein the amino acid sequence of the said peptide is selected in the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4,, which is expressed, processed, purified and prepared by recombinant DNA technology.

6. Use of a peptide derived from the scorpion or partial fragment, derivative or analogue thereof having analgesic and antitumor activity, wherein the amino acid sequence of the said peptide is selected in the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4 in the preparation of analgesic or antitumor medicament.

Patentansprüche

1. Verfahren zur Herstellung eines Peptids mit analgetischer und antitumorgener Aktivität, das aus dem Skorpion stammt, oder einem partiellen Fragment, Derivat oder Analogon desselben mit analgetischer und antitumorgener Aktivität, wobei die Aminosäuresequenz des Peptids als SEQ ID NO: 1 gezeigt ist, die aus dem Skorpion oder dem Gift des Skorpions isoliert wurde, wobei das Verfahren die folgenden Schritte umfasst:

(1) Extrahieren des Skorpions oder Skorpiongifts mit jeweils destilliertem Wasser, einer sauren Lösung und einer basischen Lösung und anschließendes Zentrifugieren zum Entfernen von Verunreinigungen,
(2) Durchführen einer hydrophoben Chromatographie mit dem Extrakt, um den Wirkstoff mit analgetischer und antitumorgener Aktivität zu erhalten,
(3) Ultrafiltrieren des Wirkstoffs mit analgetischer und antitumorgener Aktivität, um Salze und verunreinigende Proteine zu entfernen, Aufkonzentrieren und Aufreinigen des Wirkstoffs mit analgetischer und antitumorgener Aktivität mittels Kationenaustauscherchromatographie,
(4) weiteres Aufreinigen des Wirkstoffs mit analgetischer und antitumorgener Aktivität mittels hydrophober Chromatographie,
(5) Entsalzen mittels Ultrafiltration und Aufkonzentrieren und Reinigen mittels Gelfiltrationschromatographie, um einen elektrophorese-reinen Wirkstoff mit analgetischer und antitumorgener Aktivität zu erhalten,
(6) weiteres Aufreinigen des Wirkstoffs mit analgetischer und antitumorgener Aktivität mittels Umkehrphasen-Säulenchromatographie.

2. Verfahren nach Anspruch 1, wobei
das Skorpiongift in Schritt (1) mit destilliertem Wasser extrahiert wird, der Extrakt dann mit der sauren Lösung mit einem pH von 2 oder mehr und mit der basischen Lösung mit einem pH von 12 oder weniger extrahiert wird, um Verunreinigungen zu entfernen;
die hydrophobe Chromatographie in Schritt (2) zweimal durchgeführt wird, wobei als Chromatographiemedium ein Phenyl-, Octan- oder Butyl-haltiges hydrophobes Chromatographiemedium verwendet wird, der pH des Natriump-

hosphateluenten physiologisch verträglich ist und wobei die Konzentration des Phosphatrests 5 mM bis 100 mM beträgt und der Konzentrationsgradient von $(NH_4)_2SO_4$ oder Na_2SO_4 2,0 M bis 0,0 M beträgt;
das in Schritt (3) und (5) von der Ultrafiltrationsmembran zurückgehaltene Molekulargewicht 30 kDa oder 1 kDa beträgt;
das Medium der Gelfiltrationschromatographie in Schritt (5) der Chromatographie-Füllstoff Sephacryl S-100Hr, Superose 6 prep grade, Superose 12 prep grade, Superdex 30 prep grade oder Superdex 75 prep grade ist und der pH des Eluenten in einem Bereich von 2 bis 12 liegt;
das Medium der Umkehrphasen-Chromatographie in Schritt (6) der Chromatographie-Füllstoff SOURCE 15RPC oder SOURCE 30RPC ist, der Eluent 0,1%-ige wässrige Trifluoressigsäure-Acetonitril-Lösung ist und der Konzentrationsgradient von Acetonitril von 20 % bis 95 % beträgt; wobei der gesamte Prozess, welcher Extraktion, Isolierung und Reinigung einschließt, in einem halbsterilen Raum oder einem Sterilraum bei 10 °C bis 30 °C durchgeführt wird.

3. Verfahren nach Anspruch 1, wobei
der Skorpion in Schritt (1) homogenisiert und mit destilliertem Wasser extrahiert wird, der Extrakt dann mit der sauren Lösung mit einem pH von 2 oder mehr und mit der basischen Lösung mit einem pH von 12 oder weniger extrahiert wird, um die Verunreinigungen zu entfernen;
die hydrophobe Chromatographie in Schritt (2) zweimal durchgeführt wird, wobei als Chromatographiemedium ein Phenyl-, Octan- oder Butyl-haltiges hydrophobes Chromatographiemedium verwendet wird, der pH des Natriumphosphateluenten physiologisch verträglich ist und wobei die Konzentration des Phosphatrests 5 mM bis 100 mM beträgt und der Konzentrationsgradient von $(NH_4)_2SO_4$ oder Na_2SO_4 2,0 M bis 0,0 M beträgt;
das in Schritt (3) und (5) von der Ultrafiltrationsmembran zurückgehaltene Molekulargewicht 30 kDa oder 1 kDa beträgt;
das Medium der Gelfiltrationschromatographie in Schritt (5) der Chromatographie-Füllstoff Sephacryl S-100Hr, Superose 6 prep grade, Superose 12 prep grade, Superdex 30 prep grade oder Superdex 75 prep grade ist und der pH des Eluenten in einem Bereich von 2 bis 12 liegt;
das Medium der Umkehrphasen-Chromatographie in Schritt (6) der Chromatographie-Füllstoff SOURCE 15RPC oder SOURCE 30RPC ist, der Eluent 0,1%-ige wässrige Trifluoressigsäure-Acetonitril-Lösung ist und der Konzentrationsgradient von Acetonitril von 20 % bis 95 % beträgt; wobei der gesamte Prozess, welcher Extraktion, Isolierung und Reinigung einschließt, in einem halbsterilen Raum oder einem Sterilraum bei 10 °C bis 30 °C durchgeführt wird.

4. Verfahren zur Herstellung eines Peptids mit analgetischer und antitumorgener Aktivität, das aus dem Skorpion stammt, oder einem partiellen Fragment, Derivat oder Analogon desselben mit analgetischer und antitumorgener Aktivität, wobei die Aminosäuresequenz des Peptids ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, die mittels chemischer Syntheseverfahren synthetisiert, verarbeitet, aufgereinigt und erzeugt wurde.

5. Verfahren zur Herstellung eines Peptids mit analgetischer und antitumorgener Aktivität, das aus dem Skorpion stammt, oder einem partiellen Fragment, Derivat oder Analogon desselben mit analgetischer und antitumorgener Aktivität, wobei die Aminosäuresequenz des Peptids ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, die mittels rekombinanter DNA-Technologie exprimiert, verarbeitet, aufgereinigt und erzeugt wurde.

6. Verwendung eines Peptids, das aus dem Skorpion stammt, oder einem partiellen Fragment, Derivat oder Analogon desselben mit analgetischer und antitumorgener Aktivität, wobei die Aminosäuresequenz des Peptids ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, zur Herstellung eines Analgetikums oder eines Antitumormittels.

Revendications

1. Procédé pour la préparation d'un peptide ayant une activité analgésique et antitumorale dérivé du scorpion, ou d'un fragment partiel, dérivé ou analogue de celui-ci ayant une activité analgésique et antitumorale, dans lequel la séquence des acides aminés dudit peptide est présentée sous la SEQ ID NO: 1, qui est isolé du scorpion ou du venin de scorpion, comprenant les étapes suivantes :

(1) l'extraction de scorpion ou de venin de scorpion avec de l'eau distillée, une solution acide ou une solution basique, respectivement, puis la centrifugation pour éliminer l'impureté,
(2) la réalisation d'une chromatographie hydrophobe de l'extrait pour donner l'ingrédient actif ayant une activité

analgésique et antitumorale,
(3) l'ultrafiltration de l'ingrédient actif ayant une activité analgésique et antitumorale pour éliminer le sel et les impuretés protéiques, la concentration, la purification de l'ingrédient actif ayant une activité analgésique et antitumorale par chromatographie échangeuse de cations,
(4) la purification supplémentaire de l'ingrédient actif ayant une activité analgésique et antitumorale par chromatographie hydrophobe,
(5) le dessalage par ultrafiltration et concentration, et la purification par chromatographie par filtration sur gel pour donner un ingrédient actif pur à l'électrophorèse ayant une activité analgésique et antitumorale,
(6) la purification supplémentaire de l'ingrédient actif ayant une activité analgésique et antitumorale par chromatographie sur colonne en phase inverse.

2. Procédé selon la revendication 1, dans lequel
dans l'étape (1), le venin de scorpion est extrait par de l'eau distillée, puis l'extrait est extrait par la solution acide ayant un pH de 2 ou plus, et la solution basique ayant un pH de 12 ou moins pour éliminer l'impureté,
dans l'étape (2), la chromatographie hydrophobe est effectuée deux fois, le milieu de chromatographie étant un milieu de chromatographie phényl-, octane- ou butyl-hydrophobe, le pH de l'éluant phosphate de sodium est physiologiquement acceptable, la concentration de radical phosphate étant de 5 mM à 100 mM, et le gradient de concentration de $(NH_4)_2SO_4$ ou de Na_2SO_4 étant de 2,0 M à 0,0 M ;
dans les étapes (3) et (5), le poids moléculaire piégé de la membrane d'ultrafiltration est de 30 kDa ou 1 kDa ;
dans l'étape (5), le milieu de la chromatographie par filtration sur gel est le garnissage chromatographique Sephacryl S-100Hr, Superose 6 qualité préparation, Superose 12 qualité préparation, Superdex 30 qualité préparation ou Superdex 75 qualité préparation, et le pH de l'éluant est dans une gamme entre 2 et 12 ;
dans l'étape (6), le milieu de chromatographie en phase inverse est le garnissage chromatographique SOURCE 15RPC ou SOURCE 30RPC, l'éluant est une solution aqueuse à 0,1 % d'acide trifluoroacétique-acétonitrile, et le gradient de concentration de l'acétonitrile est de 20 % à 95 % ; la totalité du processus incluant l'extraction, l'isolement et la purification est effectuée en chambre semi-stérile ou stérile à 10 °C-30 °C.

3. Procédé selon la revendication 1, dans lequel
dans l'étape (1), le scorpion est homogénéisé et extrait par de l'eau distillée, puis l'extrait est extrait par la solution acide ayant un pH de 2 ou plus, et la solution basique ayant un pH de 12 ou moins pour éliminer l'impureté ;
dans l'étape (2), la chromatographie hydrophobe est effectuée deux fois, le milieu de chromatographie étant un milieu de chromatographie phényl-, octane- ou butyl-hydrophobe, le pH de l'éluant phosphate de sodium est physiologiquement acceptable, la concentration de radical phosphate étant de 5 mM à 100 mM, et le gradient de concentration de $(NH_4)_2SO_4$ ou de Na_2SO_4 étant de 2,0 M à 0,0 M ;
dans les étapes (3) et (5), le poids moléculaire piégé de la membrane d'ultrafiltration est de 30 kDa ou 1 kDa ;
dans l'étape (5), le milieu de la chromatographie par filtration sur gel est le garnissage chromatographique Sephacryl S-100Hr, Superose 6 qualité préparation, Superose 12 qualité préparation, Superdex 30 qualité préparation ou Superdex 75 qualité préparation, et le pH de l'éluant est dans une gamme entre 2 et 12 ;
dans l'étape (6), le milieu de chromatographie en phase inverse est le garnissage chromatographique SOURCE 15RPC ou SOURCE 30RPC, l'éluant est une solution aqueuse à 0,1 % d'acide trifluoroacétique-acétonitrile, et le gradient de concentration de l'acétonitrile est de 20 % à 95 % ; la totalité du processus incluant l'extraction, l'isolement et la purification est effectuée en chambre semi-stérile ou stérile à 10 °C-30 °C.

4. Procédé pour la préparation d'un peptide ayant une activité analgésique et antitumorale dérivé du scorpion, ou d'un fragment partiel, dérivé ou analogue de celui-ci ayant une activité analgésique et antitumorale, dans lequel la séquence des acides aminés dudit peptide est choisie dans le groupe constitué par la SEQ ID NO: 1, la SEQ ID NO: 3, la SEQ ID NO: 4, qui est synthétisé, traité, purifié et préparé par un procédé de synthèse chimique.

5. Procédé pour la préparation d'un peptide ayant une activité analgésique et antitumorale dérivé du scorpion, ou d'un fragment partiel, dérivé ou analogue de celui-ci ayant une activité analgésique et antitumorale, dans lequel la séquence des acides aminés dudit peptide est choisie dans le groupe constitué par la SEQ ID NO: 1, la SEQ ID NO: 3, la SEQ ID NO: 4, qui est exprimé, traité, purifié et préparé par une technique d'ADN recombinant.

6. Utilisation d'un peptide dérivé du scorpion, ou d'un fragment partiel, dérivé ou analogue de celui-ci ayant une activité analgésique et antitumorale, dans laquelle la séquence des acides aminés dudit peptide est choisie dans le groupe constitué par la SEQ ID NO: 1, la SEQ ID NO: 3, la SEQ ID NO: 4, dans la préparation d'un médicament analgésique ou antitumoral.